

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of: Ezri Peleg et al.

Application No.: 10/587,789

Attorney Docket No.: 27275.005

Filing Date: March 03, 2008

Title: HIGH LYCOPENE TOMATO  
VARIETIES AND USE THEREOF

Examiner: Worley, Cathy Kingdon  
Group Art Unit: 1638

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**DECLARATION UNDER 37 CFR 1.132**

Dear Examiner,

In order to assist in the prosecution of this application and the traversal of the Restriction Requirement subjected by the Examiner, I, Ezri Peleg, do hereby declare as follows.

**BACKGROUND**

My name is Ezri Peleg. I am a plant breeder in the Research & Development Division of Hazera Genetics Ltd. Mivhor, M.P Lachish Darom 79354 Israel. I received my M.sc. degree at the Hebrew University in Rehovot, Israel, on 1996.

My research interests and field of expertise are tomato breeding and phytopatology and crop protection.

## **PROFESSIONAL OPINION**

It is in my capacity as a plant breeder and a researcher in these fields that I offer the following comments and experimental data in the light of my reading of patent application US 10/587,789 (hereinafter referred to as the **present application**), the Restriction Requirement of July 20, 2010 and the publication of Levin et al (Theor. Appl. Genet. (2003) Vol. 106; 454-460) cited by the Examiner.

The present application claims robust tomato varieties homozygous for the *dg* mutation, producing fruit crop yield comprising an average lycopene content of at least two fold its content in currently available crop yields, wherein the plants are devoid of the *dg* linked deleterious traits. Tomato plants carrying the *dg* mutation are characterized by their dark-green fruits, which, upon ripening, become dark red, due to high lycopene content. However, the *dg* mutation is known to be linked with deleterious pleiotropic traits which include undesirable agronomical traits, which prevented the use of the high pigment *dg* gene in breeding programs (Sacks E.K. and Francis, D.M. 2001. J. Amer. Hort. Sci. 126(2):221-226).

The surprising and unexpected achievement of the present invention is that the tomato varieties of the present application, while homozygous for the *dg* mutation, do not show any of the pleiotropic and deleterious effects linked to the *dg* mutation. This is extensively claimed, disclosed and demonstrated in the application. In addition to the high lycopene concentration of the fruits, which is at least 200 ppm (claim 4 and Example 1), the tomato varieties of the present invention are devoid of the *dg*-linked deleterious traits, as specifically claimed in claims 1 and 5. In order to achieve these varieties, the high lycopene tomato lines, which contain the *dg* mutation were screened, selected for the absence of pleiotropic effects and further were crossed with commercially valuable breeding material, and stabilized for a combination of agronomically and commercially valuable traits that include: fruit crop yield, fruit quality, soluble solid content (Brix) and plant quality (steps 1-5, p. 4 of the specification). Examples 1 and 2 of the specification clearly show that the novel high lycopene hybrids of the present application are devoid of pleiotropic traits associated with the *dg* mutation, are superior over the common commercial varieties examined in production of fruits with significantly higher total soluble solids and lycopene content and have crop yield which is considered as average to high relative to a commercial

variety (Figs 1, 2 and Tables 2 and 3). Such high yield beneficial traits are a consequence of the absence of pleiotropic effects linked to the *dg* mutation in these variants.

In other words, the present invention has successfully provided, as claimed in claim 1, robust tomato varieties homozygous for the *dg* mutation, wherein tomatoes grown from these varieties have an average lycopene content at least two fold greater than in currently available varieties, while being devoid of deleterious traits associated with the *dg* mutation, when measured at peak lycopene content.

On the other hand, the homozygous *dg* tomato mutants of Levin et al (2003) (Table 1, p. 457) retain the pleiotropic, undesired traits linked to the *dg* mutation. Levin et al does not indicate, suggest or teach products with a break of the known linkage between the *dg* mutation and the undesired pleiotropic traits which include low yield, poor germination rate, brittle stems, fragile leaves, small fruits, shallow root system and premature defoliation, nor does Levin indicate, suggest or teach methods for achieving this breakage.

Experimental data showing that Levin's tomato mutants are pleiotropic are provided herewith (Appendix I). In these experiments, performed on 2002 by Hazera Genetics under my supervision, a comparison of various pleiotropic traits associated with the *dg* mutation, of LRT915 of Levin et al. (p. 457, Table 1) and the deposited hybrid HA3518 of the present application, was made. Based on repeated observations, it was found that LRT915 is pleiotropic with respect to the following traits and/or phenotypes: appearance of sensitive and fragile leaves, sudden leaf death, brittle stems, poor root system, two weeks earlier rotting fruits and lower seed germination rate (Table 1 of Appendix I). In addition, a comparison of fruit yield and lycopene content of LRT915 and HA3518 was performed at several distinct locations and environmental conditions in Israel. The results presented conclusively show that HA3518, which is an example of the tomato varieties claimed in claim 1, is superior in higher yield and higher lycopene relative to the cited strain of Levin (LRT915) (Appendix I). Furthermore, the lycopene concentration of the LRT915 tomato fruits in all locations examined was lower than 200 ppm, while the average HA3518 lycopene concentration is higher than 200 ppm, as claimed in claim 4 of the present application.

Thus, HA3518, the exemplary variety of the invention has a proven high yield, high germination rate, good fruit quality, strong root system, strong foliage, and fruits that rotten at least two weeks later than LRT915. The applicant wishes to stress that HA3518 does not suffer from the attendant pleiotropic effects found in the high lycopene prior art LRT915, and this is because the invention of the present application is the breakage of the link between *dg* and the pleiotropic traits.

To conclude, the provided experimental data support and verify the claims of the present application as filed. These results indicate that Levin's tomato mutants are pleiotropic, while the tomato varieties of the present application are superior, robust varieties devoid of the *dg*-linked pleiotropic traits.

#### Verification

I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true.

Signed:

A handwritten signature in black ink, appearing to be 'L. B. R.', written over a horizontal line.

Date: 19 September 2010